1636

PATENT

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Moya Kinnealey — Moya Kinnealey

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Anthony Giordano et al.	Art Unit:	1636
Serial No.:	09/437,458	Examiner:	Gerald G. Leffers Jr.
Filed:	November 10, 1999	Customer No.:	21559

C.P.A. filed: January 28, 2002

Title: NUCLEIC ACID SEQUENCES AND METHODS FOR IDENTIFYING COMPOUNDS THAT AFFECT RNA/RNA BINDING PROTEIN INTERACTIONS AND mRNA FUNCTIONALITY

Assistant Commissioner For Patents
Washington, DC 20231

SUBMISSION OF DECLARATION OF ANTHONY GIORDANO, PH.D.

Pursuant to 37 C.F.R. § 1.132, applicants hereby submit an executed Declaration of Anthony Giordano, Ph.D. The original unexecuted Declaration was filed unsigned on August 13, 2001. If there are any other charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: January 25, 2002

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ATTORNEY DOCKET NO. 50093/014001

Certificate of Mailing: Date of Deposit: January 23, 2002

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Assistant Commissioner for Patents
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DECLARATION UNDER 37 C.F.R. § 1.132 OF ANTHONY GIORDANO, PH.D.

1. I am an inventor for the above-referenced patent application.

2. I have read the Office Action mailed February 13, 2001 in connection with the above-referenced patent application.

3. UTRs are expected to maintain their regulatory function when operably linked to a heterologous sequence, such as a reporter gene. For example, 5' and 3' mRNA untranslated regions (UTRs) from genes of interest are commonly studied by operably linking a UTR from a gene of interest to the mRNA coding sequence of a reporter gene.

In particular, Blau et al. (WO 98/42854, published October 1998) states that "[t]he

major approach to the study of UTRs to date has been to introduce a UTR-reporter gene construct" into a cell line (page 3, lines 16 and 17). Blau also notes that a prior study demonstrated that the expression of a chimeric gene containing "the 5' regulatory region of β-actin linked to the 3'UTR of α-skeletal actin does not decline during differentiation, but increases like the endogenous α-skeletal actin gene" (page 3, lines 4-7, a copy of this reference is enclosed). Thus, the 3'UTR of α-skeletal actin maintained its regulatory role when operably linked to a heterologous sequence.

Blau also reported the ability of several 3'UTR fragments to maintain their regulatory function when operably linked to a heterologous bacterial β-glucuronidase reporter gene. Blau further notes that using the same reporter gene to study the function of several UTRs allows for a more direct comparison between the UTRs:

> The following example examines the regulatory properties of highly conserved regions (HCRs) within 3'UTRs that have retained greater than 70% homology within stretches of 100 nucleotides over 30 million years. A retroviral vector system is used within a selectable marker that allows rapid delivery of 3'UTR-reporter constructs to populations of thousands of cells within one to two weeks, avoiding problems associated with clonal analysis and long-term selection. Moreover, this vector is modular, thereby permitting direct comparison of different HCRs on gene expression, independent of 5'UTRs, promoters, protein coding regions, and polyadenylation signals. Since the 5'UTR, promoter, coding region and polyadenylation signal are constant in this vector, the influence of HCRs on gene expression can be directly compared. Ten HCRs (c-fos, c-myc, transferrin receptor, bcl2, EF1α, vimentin, ornithine decarboxylase, fibronectin, HuD, and Ran), all of which are associated with proteins with a role in growth control, were examined. Using this methods and compositions of the invention, it is shown that HCRs can cause marked changes in mRNA and protein accumulation under steady state conditions and in response to changes in the cell milieu typical of sites where tumors

> develop. Nine of ten HCRs were found to decrease mRNA stability, to different extents. Two HCRs altered mRNA translation under steady state conditions. Four HCRs mediated responses to changes in mitogen level by increasing reporter protein levels 2-fold; whereas two HCRs exhibited a 6-fold difference in their response to another environmental stress, hypoxia.

Page 27, line 25, through page 28, line 11.

Similarly, I have used polysome distribution analysis to compare the translational efficiency of an endogenous Her2 mRNA gene to the translational efficiency of a chimeric mRNA molecule containing both the 5'UTR and 3'UTR of Her2 operably linked to a luciferase reporter gene (U.S.S.N. 60/278,902; filed March 26, 2001). A chimeric Her2 UTR/reporter construct encoding this chimeric mRNA was stably transfected into the MCF7 breast cancer cell line. The resulting polysome distribution of the chimeric mRNA was similar to that of endogenous Her2 (Figures 1B and 1D containing this data from U.S.S.N. 60/278,902 are attached). This result indicates that the 5'UTR and 3'UTR of Her2 maintained their regulatory activity when operably linked to the luciferase heterologous sequence.

The 5'UTR and 3'UTR of Her2 were also shown to modulate the half-life of the chimeric mRNA molecule (U.S.S.N. 60/278,902). In MCF7 cells stably transfected with a control luciferase construct that did not contain any HER2 UTRs, the half-life of the luciferase mRNA was approximately 4 hours. In contrast, the mRNA corresponding to the stably transfected luciferase construct containing both 5' and 3' HER2 UTRs had a half-life of only approximately 1 hour. These data demonstrate that UTRs operably

linked to heterologous coding sequences can modulate mRNA stability.

4. Heterologous sequences, such as reporter genes, can be readily identified by one skilled in the art. Examples of commonly used reporter genes include chloramphenicol acetyltransferase, firefly luciferase, renilla luciferase, β-galactosidase, secreted alkaline phosphatase, human growth hormone, β-glucuronidase, green fluorescent peptide, and red fluorescent protein, among others (see, for example, Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, 1995).

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: 8/23/01

Anthony Giordano, Ph.D.